UNIVERSITY OF GONDAR

COLLEGE OF MEDICINE AND HEALTH SCIENCE

SCHOOL OF PHARMACY

DEPARTMENT OF PHARMACOLOGY

EVALUATION ON ANTI ULCER EFFECT OF HYDROMETHANOLIC CRUDE EXTRACT AND SOLVENT FRACTIONS OF THE STEM BARK OF *FICUS THONNINGII (MORACEAE)* ON RODENT MODELS

A THESIS SUBMITTED TO THE DEPARTMENT OF PHARMACOLOGY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN PHARMACOLOGY

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GONDAR, ETHIOPIA

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This is to certify that the thesis prepared by Habtalem Adane, entitled: "**Evaluation on anti-ulcer effect of Hydromethanolic crude extract and solvent fractions of the stem bark of *Ficus thonningii (Moraceae)* on rodent models** "and submitted in partial fulfillment of the requirements for the degree of master of science in pharmacology complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of Variance
Aq.EFT	Aqueous Extract of *Ficus thonningii*
CEFT	Crude Extract of *Ficus thonningii*
EGF	Epidermal Growth Factor
FT	*Ficus thonningii*
IAEC	Institutional Animals Ethics Committee
NSAIDs	Non-Steroidal Anti-Inflammatory Drug
OECD	Organization for Economic Co-operation and Development
PGE2	Prostaglandin E2
PL	Pylorus Ligation
PPIs	Proton Pump Inhibitors
PUD	Peptic Ulcer Disease
ROS	Reactive Oxygen Species
SEM	Standard Error of Mean
SPSS	Statistical Package for Social Science
UI	Ulcer Index
UP	Percent of animals with Ulcer
US	Ulcer severity Score

ABSTRACT

Introduction: Peptic ulcer disease is the most predominant gastrointestinal diseases.The current treatment strategies have multiple drawbacks due to side effects and complexity of administration.An Endeavour to search an ideal anti- ulcer agent from medicinal plant reports is going on. In the present study, we investigated the anti -ulcer effect of hydromethanolic extract and solvent fractions of *F. thonningii* stem bark.

Objectives: To evaluate the anti-ulcer effect of *F.thonningii* stem bark on ulcer induced rodent models.

Methods:*F.thonningii* stem bark was collected and the shed dried plant material was macerated and extracted by 80% hydromethanol.Part of the dried crude extract was further fractionated with different solvents.Hydromethanolic crude extract was used for acute toxicity test,phytochemical screening and antiulcer activity test on pylorus ligation and indomethacine induced ulcer model. While, the time dependent study on ethanol induced ulcer was evaluated by both the crude extract and solvent fractions. Preliminary phytochemical screening was done based on standard procedures.Acute toxicity study was carried out on Swiss albino mice.

Results: Preliminary phytochemical screening showed the presence of: Saponins, Flavonoids, Terpinoids, Glycosides, Anthraquinones, Tannins, Alkaloids and Phenolic compounds. The extract showed significant reduction in Total acidity (CEFT 100,200,400mg/kg) ($P<0.001$);Gastric volume (CEFT 100) ($P<0.01$);(CEFT 200,400mg/kg) ($P<0.001$), pH (CEFT200,400 mg/kg)($P<0.05$) and Ulcer Index (CEFT 200,400mg/kg) ($P<0.05$) as compared to the negative control. The crude extract and aqueous fractions of *F.thonningii* 200 mg/kg showed significant reduction on ulcer index ($P<0.05$) on repeated dose administered groups of ulcerated animals induced by ethanol. Ulcer healing effect on indomethacine induced ulcer were not significant ($P>0.05$) for all three doses (CEFT100, 200,400 mg/kg). No sign of toxicity was observed up to the limit dose (2000mg/kg).

Discussion and Conclusion: This study demonstrated *F.thonningii* stem bark has a potential anti-ulcer activity that might be due to anti-secretory or cytoprotective effect of the plant's phytochemicals.

Key Words: Ethanol, *F.thonningii,* Pylorus Ligation, Solvent fractions, Ulcer healing.

1. INTRODUCTIONON

1.1 BACKGROUND

Peptic ulcer disease is an erosion in a part of the gastro intestinal mucosa, typically in the stomach (gastric ulcer) or first few centimeters of duodenum (duodenal ulcer) that may pass through the muscularis mucosa(1).

It is the most prevalent gastrointestinal disease with a worldwide prevalence of about 40% in developed and 80% in developing countries (2).In United States alone, the annual cost associated with peptic ulcer disease is estimated to be $6 billion. It can also leads to gastric cancer which kills over 700000 people per year globally. Although most studies reported that a decrease in the incidence or prevalence of GI disorders over time due to a decrease in *H. pylori* associated cases, peptic ulcer disease remains a common condition (3) as it was reported with point prevalence of 59.6% in Bangladesh (4) and 33.4% in Brazil (5).In Ethiopia,PUD is highly associated with *H. pylori* infection, alcohol intake and age (6-8).It is the leading among the top Ten diseases of Hospital admission In south Africa (9).Complications of peptic ulcer disease have also severe health and economic burden globally that leads to prolonged hospital stay for patients and therapeutic management complication for health care workers. Such as perforation which becomes a global emergency with mortality rates of 30% (10) ,Hemorrhage,obstruction,pyloric stenosis (late complication characterized by lots of vomiting), lymphoma and gastric cancer due to *Vac A* and *Cag A* strain of *H. pylori* and even risk of osteoporosis for those treated with PPIs (11)**.**In Kenya, perforated peptic ulcer was reported mainly on young males (12)**.**In Tanzania,Gastritis (61.10%), Gastro esophageal Reflux Disease(57%),Peptic Ulcer Disease (24.1%) and Gastric cancer (6.7%) are the leading causes of dyspepsia (13).In Ethiopia, a report in Karamara Hospital showed 78 from 92 patients were admitted with gastric outlet obstruction secondary to PUD (14).Another report in Tikur anbessa Hospital indicated patients with Complicated PUD comprised 3.8% of the total major surgical procedures (15) and 6.7% of hospital admission in St.Poul Hospital (16).

1.1.1 Etiology of Peptic Ulcer Disease

Peptic ulcer disease occurs due to an imbalance between mucosal damaging (acid, pepsin) and protecting (mucus, bicarbonate, Prostaglandin E2and I2) mechanisms. Acid secretion is a physiologically useful process of the stomach as gastric acid induces pepsinogen activation to initiate digestive process and kills microbes and ensuring a stable intra-gastric environment. Endogenous secretagogues (positive regulators) of acid secretion are Acetylcholine, Histamine and Gastrin. While Prostaglandins (PG E2 and I2) act as negative regulators of acid secretion. *Helicobacter pylori* infection is the major Risk factor (approximately 80%) (17) and its Eradication reduces the incidence and relapse rate of ulcers(7, 18-20). NSAIDs,Steroids , Smoking , Alcohol , Stress and Depression are also etiologic agents for PUD (21).Sometimes, Acid hypersecretion due to Zollinger-Ellison-gastrine secreting tumor (gastrinoma) is also responsible for multiple ulcers. Even, treatment with high dose of PPI or curative resection leads to loss of negative feedback on acid secretion, increase histamine production and abnormal gastric emptying (too fast can give duodenal ulcers and too slow can cause gastric ulcers).Some unavoidable factors are also associated with gastric ulcer:Sex,age, ethnic backgrounds (African- Americans or Hispanics have 2-fold higher),Chronic diseases such as Congestive Heart Failure, blood group(type O),Genetics (*Pepsinogen C* gene polymorphism), radiation damage and viral infections are the main causes for PUD (22).

1.1.2 Pathophysiology of Peptic Ulcer Disease

Physiologically, gastric mucosal barrier is composed mainly of protective luminal mucus layer, gastric epithelial barrier, immune cells, gastric microcirculation and sensory gastric innervations**.** When gastric mucosa gets exposed to damage by gastric acid or other irritating chemical, afferent neurons are activated and directly start controlling the tone of the sub-mucosal arterioles, which regulate mucosal blood flow. When sensory afferent nerves of the superficial mucosa detect gastric acid, they respond by releasing neurotransmitters as *substance P* and *calcitonin gene-related* peptide. These mediators cause relaxation of smooth muscle surrounding gastric mucosal arterioles, resulting in elevation of mucosal blood flow. In addition, vagal activation increases mucus secretion, while nervous response to stress control central *corticotropin-releasing factor* signaling pathways. Furthermore, the transient

receptor potential *vanilloid*-1 agonists are effective in protecting gastric mucosa against various experimentally induced ulcer models(22).

The stomach mucosa has two important types of tubular glands: oxyntic and pyloric glands. The oxyntic glands secrete hydrochloric acid, pepsinogen, intrinsic factor and mucus. The pyloric glands secrete mainly mucus for protection of the pyloric mucosa from the stomach acid. They also secrete the hormone gastrin. A typical stomach oxyntic gland is composed of three types of cells: (1) Mucus neck cells, which secrete mainly mucus*;* (2) peptic (chief) cells, which secrete large quantities of pepsinogen; and (3) parietal (oxyntic) cells, which secrete an isotonic solution of essentially pure hydrochloric and intrinsic factor. The pH of this solution is as low as 0.8, the concentration of H^+ being a million times higher than that of plasma. *Carbonic Anhydrase* enzyme abundantly present in the gastric parietal cell combines carbon dioxide and water forming carbonic acid, from where bicarbonate ion (HCO_3^-) is exchanged with plasma Cl^-. H^+ ion is pumped out against the concentration gradient into the gastric lumen by *H^+/K^+ATPase* that is located in the apical membrane of the parietal cells. This pump generates the largest ion gradient in vertebrates, with an intracellular pH of about 7.3 and an intra-canalicular pH of about 0.8.Parietal cells contain receptors for Histamine, Gastrin, and Acetylcholine (23). Gastrin is secreted by endocrine cells in the gastric antrum and duodenum. Pepsinogen, the inactive precursor of pepsin, is secreted by the chief cells located in the gastric fundus. Pepsin is activated by optimal acidic pH of 1.8 to 3.5, inactivated reversibly at pH 4, and irreversibly destroyed at pH 7 (24). Histamine activates histamine-2 receptors on the acid-producing parietal cells to stimulate acid production. In addition, any imbalance in the activity of antioxidant enzymes like Superoxide dismutase (SOD), Catalase (CAT) and Gluthathione peroxidase (GPX) may lead to faulty disposal of free radicals which leads to gastric mucosal damage and ulcer. Disturbance of apoptosis balance, bile salts that destroy the permeability barrier of gastric mucosa and increase mucosal permeability to acids. Any disturbance in ulcer healing process also leads to peptic ulcer disease. Ulcer healing process includes inflammation, cell proliferation, re-epithelialization, formation of granulated tissue, angiogenesis, interactions between various cells and the matrix and tissue remodeling, all these events are controlled by Cytokines, Growth factors *(EGF)*,Gastrointestinal hormones (*Gastrin*,*CCK)*and *Orexigenic* peptides (*ghrelin*,*orexine*-A and *obestatine),*Transcription factors, as well as COX-2 are important for

epithelial cell proliferation and reconstruction of gastric glands during gastric ulcer healing. Nitric oxide, Endothelin, Prostaglandins and Metalloproteinase's are important for vascular remodeling and mucosal regeneration within gastric ulcer(25, 26)**.**

1.1.3 Diagnosis of Peptic Ulcer Disease

Peptic ulcer disease can be diagnosed by different techniques starting from patients history, physical examination for signs and symptoms which consist of a sensation of pain or burning in the epigastrium, early satiety (inability to finish a normal-sized meal),fullness during or after a meal, or a combination of these symptoms. The true incidence of peptic ulcer depends on satisfactory case-history data; and- good diagnostic facilities. Since diagnostic investigations are limited in most African countries, incidence reports are not fully certain. In addition, laboratory tests are used for diagnosis of PUD.Urea Breath Tests require the ingestion of urea labeled with the nonradioactive isotope carbon 13 or carbon 14 with Specificity and sensitivity approaching to 100%. Cost and inconvenience are disadvantages of this test. Stool Antigen test using monoclonal antibodies are as accurate as urea breath tests and it is cheaper also. But, like urea breath test, it detects only active infection. Serologic Antibody testing detects immunoglobulin G specific to *H. pylori* in serum since antibody can stay for long time in the body. But, this test cannot distinguish between an active infection and a past infection. Endoscopy with biopsy is recommended to rule out cancer and other serious causes especially in old patients. Culture and Polymerase Chain Reaction can allow for susceptibility testing but are not readily available for clinical use (27).

1.1.4 Management of Peptic Ulcer Disease

Based on the involvement of multiple factors in peptic ulcer, several therapeutic strategies have been adopted against it. These, include suppression of the aggressive factors with use of antacids, specific antagonists of muscarinic -M receptors, Gastrin receptors, Histamine-H2 receptors, Proton Pump Inhibitors (PPIs), and mucoprotective agents, antimicrobials for eradication of *H. pylori* and analogues of prostaglandins. Researches for development of antiulcer agent, aims to address one or the other of these issues (28).

1.1.4.1 Non Pharmacological Approaches

Several studies implied that modulating life style factors as dietary factors, controlling stress, reducing smoking, physical exercises and alcohol intake may directly prevent the initiation of gastric ulcers. Weight loss, avoid precipitants, raise the head of the bed, not eating late at night, stop NSAIDs/steroids, Diet therapy, caloric distribution, and micronutrients, such as vitamin A, Zinc, Selenium, and Vitamin C promote healing in especially during the recovery phase. In addition, vitamin C has a beneficial effect in eradication of *H. pylori*. Fibers and probiotics also play a role by reducing side effects of antibiotics and treatment time (29, 30)**.**

1.1.4.2 Conventional Anti-ulcer Agents

The goals of treating peptic ulcer disease are to relieve pain, heal the ulcer and prevent ulcer recurrence (28)**.**Antacids, H2-receptor antagonists are effective in healing both gastric and duodenal ulcers. PPIs (Omeprazole, Pantoprazole) inhibit gastric acid by blocking the H+/K+-Adenosine Triphosphatase enzyme system.PPIs are the most effective drugs to treat PUD. But,there are still areas of medical need in acid controls that are not met by PPIs as these drugs are slow to achieve steady state inhibition (typically take 3 to 5 days); Inter-individual variations in efficacy due to CYP2C19 polymorphism and the requirement of meal time dosing to ensure adequate levels of the drug during periods of H^+/K^+-ATPase activation. In order to avoid the limitations encountered on PPIs, the search for new drugs is still an active area of scientific research. Such scientific researches end up with the development of more potent gastric acid suppressants called potassium competitive acid blockers (P-CABs) which have several advantages over PPIs as:P-CABs rapidly achieve therapeutic plasma levels and provide almost complete inhibition of gastric acid secretion; No inter-individual variation; P-CABs action is independent of secretory state;P-CABs are acid stable and do not require an enteric coating formulation. Some of these agents are, SCH28080, Revaprazan(only used in South Korea and India) and Vonoprazan (recently approved) in Japan (31)**.**

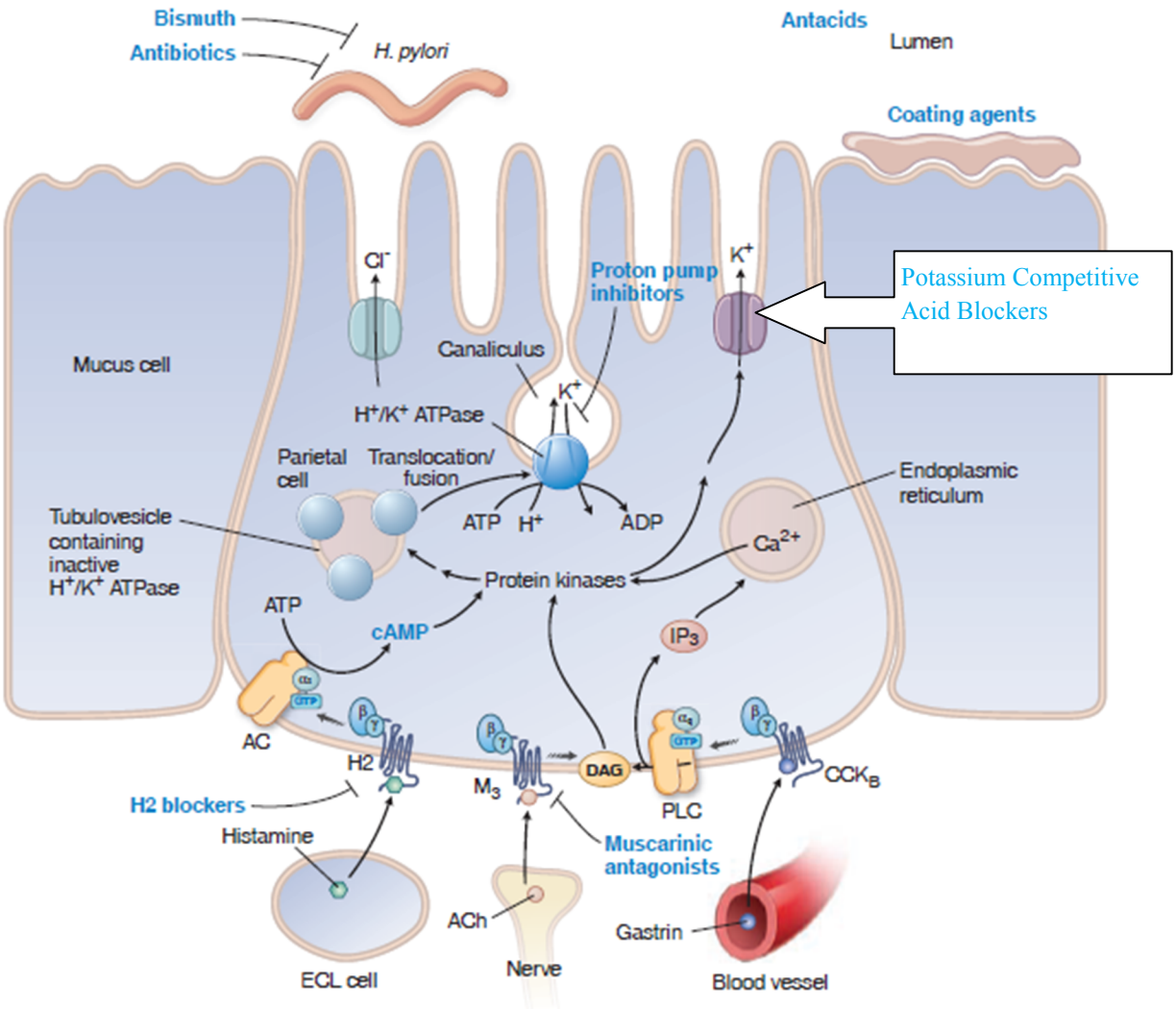

Figure 1: Sites of action of drugs used to treat peptic ulcer disease(24).

1.1.4.3 Miscellaneous Investigational Anti-ulcer Agents

Currently, different studies have been undertaken to find an ideal investigational anti -ulcer treatment by various agents as drugs of other uses, metabolic products, minerals and growth factors with different mechanism of actions (32)**.**Tacrolimus has an ulcer protective activity through its anti-secretory, antioxidant and anti-inflammatory action and its inactivation of immune cells which binds to the *FK506 binding* protein and this complex interacts with *calcineurin* which inhibits the catalytic activity of calcineurin. Nitric Oxide (NO) inhibits gastric secretion by suppression of histamine release from *Enterochromaffin-like Cells* and inhibits platelet aggregation. Furthermore, NO and *Peroxynitrite* have a direct toxic effect on

H. pylori VI strain. Copper, as a cofactor and a prosthetic component of several cuproenzymes controlling oxidation-reduction reactions including Cytochrome c- oxidase, Superoxide dismutase has ulcer protective effect. Recently, probiotics have been reported to have antiulcer effects especially those of *Saccharomyces Boulardii* yeast or lactic acid bacterial species which have ability to eradicate *H. pylori* bacteria (33)**,**protect gastric mucosal barrier, upregulate prostaglandins, mucus, growth factors and anti-inflammatory cytokines, Increase cell proliferation to apoptosis ratio and induce angiogenesis. In addition, some advanced procedures as Transplantation of bone marrow mesenchymal stem cells or possibly gastric epithelial stem cells are also a proposed for the treatment of gastric ulcers that requires further investigation (34)**.**Carbon monoxide (CO) is also involved in the restoration of murine gastric epithelial cells, this is because of CO solution accelerates the gastric ulcer healing by promoting re-epithelialization and cell migration through the activation of protein kinase C (PKC) which is proposed to be a novel therapeutic approach for the treatment of gastric mucosal injuries in the future(32).

1.2 LITRATURE REVIEW

1.2.1 Traditional medicines

Peptic ulcer is one of the world's major gastrointestinal disorders and affecting 10% of the world population and 75–80% of the world populations still use herbal medicine mainly in developing countries, because of better cultural acceptability and lesser side effects especially, The East African region is well known with plant biodiversity with an estimated of over 21 000 higher plant species. This provides Ethnopharmacologists for phytochemical and pharmacological studies (35) in different areas based on the plants phytochemical constituents and traditional claims as plant metabolites like Flavonoids and Tannins have active principles of antiulcer activity (36) by correlating the scientific world and the traditional medicine's close relation with the community's health care system. Ethiopia is enriched in both medicinal plant biodiversity and cultural knowledge on ethno pharmacology of medicinal plants (37, 38) which are the integral part of the variety of cultures over centuries for human (39) and livestock medicines (40).In Ethiopia, where major way of indigenous knowledge transfer on medicinal plants, was by word of mouth to a family member,none of the traditional healers had written documents on traditional medicines (41)**.**So, several ethno botanic studies reported in different areas can play a great role for the documentation and preservation of medicinal plants and the knowledge itself as well as further screening studies (42, 43).

1.2.2 Herbal Medicines for peptic ulcer disease

Large numbers of studies have been done on the relationship between plant extracts and their anti-ulcer activity on animal experimental models.Though selecting a suitable model been difficult as each model has its own advantages and disadvantages, one can select based on the resources and objectives of study. Generally, Pyloric Ligation, Alcohol induced ulcer, Cold stress induced ulcers and Indomethacine induced ulcer models are some of them. The plants' families majorly include; *Moraceae* (family of *F.thonningii*), Portulacaceae and Urticaceae (44).A medicinal plant*, Ipomoea-batatas* increased gastric anti-oxidant enzymes on asprine induced and cold stress induced ulcer models (45) Another medicinal plant *Aloe vera* was also reported gastroprotective activity on ethanol induced ulcer models (46) a single dose 250mg/kg of *Artocarpus Integrifolia* screened by pylorus ligation model was reported

significant reduction of gastric juice volume,total acidity and pH with 50.9% reduction in ulcer index (47) a 100mg/kg,200mg/kg and 400mg/kg *Centelia asiatica* has been reported ulcer protecting effects on ethanol induced ulcer model (48) anti-ulcer effect of *Citrullus colocynthis* on pylorus ligation model was reported comparable with the standard drug ranitidine (49) there are many medicinal plant species studied in different ulcer models are*:Ocimum sanctum*(50);*Glycyrrhiza glabra*on (51);*Hedranthera barteri* (52)**;***Ocimum suave* (53)**;***Swietenia mahagoni* (54).*F.sycomorus* L.(55) and *F.sreligiosa* (56).

1.2.2 The Experimental Plant: *Ficus thonningii Blume (Moraceae)*

Ficus thonningii Blume also known as the common wild fig (Eng) is grouped in a family of *Moraceae.*It is a multi-stemmed, evergreen or briefly deciduous tree. It mainly distributes in upland forests of tropical and sub-tropical Africa. The bark is usually smooth, pale to dark grey with vertical lenticels and the tree usually has aerial roots that hang from branches.Globally,nearly 750 species of ficus were found (57).*F.thonningii* has been described as a complex of taxa under which *F.rokko*, *F.rhodesiaca*, *F.burkei*, *F.petersii*, *F.persicifolia* and a number of other *Ficus* species are all lumped (58).The different local names of the plant are: jammeiz al abiad (Arabic);India-laurel fig (French); mtschamwa (Swahili).In Ethiopia, *F. thonningii* is called shibaka (Tigrigna) and Chibha (Amharic)(59).

Figure 2: Photograph of *F.thonningii Blume Wild (Moraceae)*

F.thonningii has anti-malarial activity both in vitro and in vivo according to the report in Nigeria (60)**.***F.thonningii* contains different classes of phytochemicals as: Flavonoids,

Tannins, Soluble starches,Glycosides,Alkaloids, Terpenoids and Essential oils on the stem,bark, leaves and roots. Furthermore, identified chemicals as orientin, vitexin and isovitexin and stilbenes which include resveratrol, resveratrol glucosides and stilbenes glucosides which have anti-ulcer activities are also identified (58).

F. thonningii is extensively used by ethno medical practitioners for treating various ailments in Africa including analgesic, antimicrobial, anti-helmintic, antioxidant, cardio protective, hypotensive and hypoglycemic effects of the plant extracts. In addition, it is used for infertility, impotence, yellow fever, loss of appetite (root), diarrhea (chewing the root), and stomachache (chewing the inner bark).In Nigeria, a maceration of the leaves is used for treating stomach pains, gastritis, gastric ulcers and colitis. Traditionally, the stem bark is pound and the infusion is used for treating influenza, sore throat, colds, arthritis, rheumatism and to relieve inflammation (61).

F. thonningii stem bark decoction is also used in Mali and Senegal, to treat respiratory diseases such as;pneumonia, bronchitis, emphysema. and for treating diarrhea, cysts, skin diseases and ulcers in Ethiopia (59).In addition, *F. thonningii* exhibit trophic developmental changes on the neonatal gastric tissues without any adverse effects (58).

1.3 JUSTIFICATION OF THE STUDY

Globally, Peptic ulcer accounts approximately 1% mortality (23). In many poor countries, expenditures for drugs range from 20% to 60% of total budget on health. Drug resistance has also a startling impact on the cost of curing patients(62).Due to this, catalyze research and innovation to speed the development of resistance-fighting technologies has been set as a recommendation. The rise of resistant strain of *H.pylori* on different Antimicrobials especially in developed countries has been given great attention (63).Patients on once daily doses of PPIs failed at a rate of 56% Which indicates significant PPI drug resistance and rapid metabolism due to CYP2C19 polymorphism (64, 65).

The expected increase in the cost of commercial drugs and side effects of drugs as Cardiovascular,Endocrine,and Sedative effects, complexity of administration,drug-drug and drug-food interactions generally leads to find new chemicals for ulcer treatment (66).The search for new drugs species have been going on. Availability of secondary metabolites which have anti-ulcer activities indicates the anti-ulcer potential of *F.thonningii* that is

repeatedly reported throughout different parts of the world but no report was made on the antiulcer activity of hydro methanolic and solvent fractions of the stem bark of the plant. Due to this, the study was done and verified the claims of the previous reports on the knowledge of native traditional medicine practitioners.

The finding of this experimental study helps the scientific community to further investigate on this medicinal plant by initiating advanced studies on formulations and molecular mechanisms of plant source drugs and by isolating a specific antiulcer compound.

2. OBJECTIVES

2.1 General Objective

To evaluate the antiulcer activity of Hydromethanolic crude extract and solvent fractions of the stem bark of *F. thonningii (Moraceae)* on rodent models.

2.2Specific Objectives

- To undertake preliminary phytochemical screening on *F. thonningii* stem bark.
- To evaluate anti-ulcer effect of Hydromethanolic extract of *F. thonningii* stem bark on the dose dependent pyloric ligation ulcer model.
- To evaluate the time dependent anti- ulcer effect of Hydromethanolic crude extract and solvent fractions of *F. thonningii* stem bark on ethanol induced ulcer model.
- To determine acid reduction ability of the crude and solvent fractions of bark of *F. thonningii* plant extract using colorimetric and titrimetric analysis.
- To evaluate the ulcer healing effect of crude extract on indomethacine induced ulcer model.
- To determine the acute oral toxicity effect of Hydromethanolic extract of the stem bark of *F.thonningii*

3. MATERIALS AND METHODS

3.1 Materials

3.1.1Equipments, Drugs, Chemicals and Supplies

Methanol (ReAgentchem.Ltd;India),n-hexane (bululuxlab),Chloroform (NICELab.),Distilled water,Dethylether(BlululuxLab.),0.1NNaOH(CentralDrugHouse,India),HCl,sulfuricacid,Tween 80 (Bululux;Lab.),Phenolphthalein (RFCL;RANKEM,India),Analytical graded Balance,Lenses (10X),pH meter,Absolute Ethanol (96%)(Bululux lab.),Mayer's Reagent,Normal Saline ,Ranitidine (ACILOC,Cadila;Ethiopia),Misoprostol 200 mcg tablet (Jai Pharma Ltd),10% buffered formaline.Mayer's reagent (Mettle-Toledo Ltd, Switzerland),Glacial acetic acid (Lobe chemi, India),2% Ferrous and Ferric Chloride,10% NaOH,Conc.H2SO4,diluted Ammonia. Filter paper (Watt man no.1EXACL; India).All chemicals were analytical grade purchased from Pharmaceutical Fund and Supply Agency,Gondar-Ethiopia,and obtained from Department of Pharmaceutical Chemistry,Pharmacognosy and pharmacology, University of Gondar.

3.1.2Collection of Plant Material

Stem bark of *F. thonningii* was collected from the local area of Gondar City Ethiopia, in January 2017.The plant was duely identified and authenticated by a Botanist and specimens were deposited at the herbarium in University of Gondar with voucher number of (HA001).The collected plant bark was washed immediately with running water and shade dried at room temperature, powdered mechanically and become ready for extraction.

3.1.3 Experimental Animals

White Swiss albino Mice (20-38mg) and Wistar Rats (150 – 250g) of either sex were used in this experiment. Animals were obtained from animal houses of departments of pharmacology and faculty of Veterinary Medicine University of Gondar, and National Veterinary Institute Bishoftu, Ethiopia. Animals were housed in standard cages at room temperature and 12:12 hours of light dark cycle. Animals were fed with standard pellet diet and water *ad libitum*. Experimental protocol for care of animals was undertaken as per guidelines for use of laboratory Animals (67).

3.2 Methods

3.2.1 Preparation of Hydromethanolic Bark Extract

The stem bark of *F. thonningii* was shade dried and reduced to coarse powder then grinded to coarse powder using mortar and pestle. 1 kg of the powdered material was first extracted by maceration with an Erlenmeyer flask using 80% Methanol in distilled water for a period of 3 days with occasional shaking. Then, the extract was initially filtered by gauze (muslin) and re-filtered by electrical sucker using filter paper (whatman no.1, UK). The marc was re-macerated for the second and third times with fresh hydro alcohols, for a total of 9 days. After filtration, the three extracted solutions were combined and concentrated in hot air oven adjusted to a temperature of 40 ^{0}C. In addition, Rotary evaporator (RE200, Germany) was used under reduced pressure to concentrate and separate methanol from the distilled water in department of Organic Chemistry, University of Gondar. It was then put into desiccator to prevent moisture absorption. The crude extract was used for acute toxicity, preliminary phytochemical screening and anti- ulcer activity testing (68). The percentage yield of crude extract was calculated as:

Percentage yield = (Weight of extract/Weight of sample) X 100

3.2.2 Fractionation

N-hexane, Chloroform and distilled water were used as solvents for fractionation. Selections of organic solvents were carried out based on their increasing polarity index and immiscibility with water. Half of the above 80% hydro methanolic extract was used. First, 68 grams of the crude extract was weighed and dissolved in 200 ml of distilled water. Then the suspension was poured in to a separatory funnel. Then, by adding 150 ml of n-hexane each time 3 times, the mixture was shaken and filtered to obtain the n-hexane fraction. The density of n-hexane is less than water and so formed the top phase. Likewise, 150 ml of chloroform was added and shaken gently. Because the density of chloroform is greater than water and also immiscible, it forms two phase with the bottom for chloroform fraction and the top for aqueous phase. Chloroform was added three times and filtered to obtain the chloroform fraction. The final marc left after was filtered and aqueous fraction was taken. Then, all fractions were concentrated in hot air oven adjusted to a temperature of 40˚C (69) to remove

the solvents. all fractions were placed in a desiccator. Finally, the dried fractions of each solvent were collected except the n-hexane fraction which was not sufficient for further tests.

3.2.3 Phytochemical Screening

Preliminary Phytochemical screening tests was carried out to determine the major classes of phytochemicals on crude extract of *F. thonningii* stem bark by using different standard test procedures(70-72).

Terpenoids: Salkowski's test: 2 ml of chloroform was added to 0.5 g of plant extracts and 3 ml of concentrated sulphuric acid resulting in the formation of two layers. A reddish brown color at the interface of these layers should be shown for confirmation of the presence of Terpenoids.

Saponins*:* Froth test**:** A milliliter of plant extract filtrate has been shacked in 10 ml of distilled water for 30seconds.a Persistent frothing indicated the presence of Saponins.

Flavonoids*:* Ferric Chloride test: in the presence of Flavonoids, few drops of ferric chloride solutionon the Test solution will show intense green color.

Test for Tannins: The plant extract was stirred with 2 ml of distilled water and filtered with filter paper (What man No. 1) few drops of 2% ferric chloride were added to the clear filtrate. Then, the filtrate was observed when it gives a green precipitate indicating the presence of tannins.

Tests for Steroids;2ml extract was dissolved in 2 ml of chloroform & equal volume of concentrated H2SO4 acid was added from the side of test tube .a red color formed in the lower chloroform indicates the presence of steroid (70).

Identification test for alkaloids: Few drops of Mayer's reagent were added to1 ml of the powder of plant extract. a whitish opalescence precipitate with Mayer's reagent indicated the presence of alkaloids(71).

Phenolic compounds: Ferric chloride test**:** Small amount of extract/fraction was dissolved in distilled water and to this few drops of neutral 5% ferric chloride solution was added. Formation of blue green color indicates the presence of Phenolic compounds(72).

Test for Glycosides: keller-killiani test:1 drop of ferric chloride was added on 2 ml crude extract and 2 ml glacial acetic acid. The mixture was then poured in to a test tube of 1 ml conc.H2SO4. Formation of a brown ring indicates the presence of glycosides.

Test for Anthraquinones: 3 ml of the plant extract was dissolved by benzene and filtered with watt man filter paper .Then 2 ml of Ammonium hydroxide was added. Formation of purple ring indicates the presence of Anthraquinones.

3.2.4 Acute Toxicity Test

Acute toxicity study was carried out using the guidelines described by OECD-425 ((67).Five female albino mice were selected Randomly and fasted for 4 hrs but water was allowed. A limit dose 2000 mg/kg of *F.thonningii* Stem bark crude extract was administered sequentially for three mice and animals were observed individually for behavioral profile (alertness, restlessness, irritability and fearfulness), autonomic profiles (defecation and urination), neurologic profile (spontaneous activity, reactivity, touch response, pain response and gait), physical states as lacrimation, loss of appetite, tremors, hair erection, salivation, diarrhea and for morbidity or mortality after dosing continuously for two hours, periodically during the first 24 hours and daily thereafter. Body weight of the five mice was measured initially at the time of administration, at the seventh and fourteenth day of administration with the remaining two controls. Since all three animals were survived, the LD50 is greater than the limit dose and the test was terminated (i.e. carried to full 14-day observation without dosing of further animals) (67).

3.2.5 Grouping and Dosing of Animals

The anti-ulcer activity of *F.thonningii* was studied using three experimental models of ulcer and grouping of animals was done randomly to different groups each consisting of six animal. The test groups received different doses of the plant extract. The negative control groups were treated with solvents of the extracts only while the positive control groups were treated with Ranitidine 50 mg/kg and Misoprostol 5µgm/kg.Animals which came from other places were allowed to acclimatize to the laboratory conditions for one week and had free access to tap water and Normal Pellet Diet (NPD) until they were assigned to individual groups.

3.2.5.1 Pylorus Ligated Induced Ulcer model

The shay rat model with some modification which was used by previous studies was applied (47, 73)**.**Rats were randomly divided into five study groups, each consisting of six animals.

Group 1 was the negative control group, which was received vehicle only (distilled water + 6% Tween 80). Group 2 was served as a positive control and rats ware pretreated with Ranitidine 50 mg/kg for ten days. Groups3, 4 and 5 were received100, 200 and 400 mg/kg of hydromelthanolic extracts of *F.thonningii*.

During the study, rats were fasted for 24 h before the study, but had free access to water till the last 4 hours. After 1h of the last drug treatment, animals were anaesthetized inhalationally with diethyl ether and the abdomen was opened by a small midline incision below the xiphoid process. Pyloric portion of the stomach was lifted out and ligated carefully to avoid traction to the pylorus or damage to blood supply of gastric mucosa. The stomach was replaced carefully and the abdominal wall was closed by interrupted sutures. After four hours of pyloric ligation, Rats were sacrificed by inhalational anesthetic ether. The abdomen was opened, cardiac end of the stomach was dissected out and the content was drained into a test tube. The gastric juice was collected and centrifuged at 1000 rpm for 10 minute the volume of the supernatant was noted and taken for the determination of Total acidity and pH.The stomach Mucosa of each animal was washed with saline and running water, labeled and placed on sodium phosphate buffered 10% formalin until it was examined for lesions by using hand lens (10X) and scored accordingly.

3.2.5.2 Ethanol-Induced Ulcer Model

The ulcer was induced by administering ethanol following the method by Hollander D. et al (74) and previous studies to determine the anti-ulcer effect of repeated and single dose administration with some modifications (75, 76)**.**Animals were randomly assigned to 10 groups each consisting six Animal. Group 1and 6 received the vehicle (distilled water+4% Tween 80 for single dose and repeated dose for 10 days respectively) and considered as a negative control, whereas groups 2 and 7 were served as a reference standard and pretreated with Misoprostol 5µg/kg (single and repeated for 10 days respectively). Groups 3, 4 and 5 were treated with 200 mg/kg (crude, chloroform and aqueous fractions in single dose respectively) and 8,9 and 10 were pretreated with 200 mg/kg of crude extract, chloroform and aqueous fractions of *F.thonningii* for 10 days respectively. Animals were fasted for 24 hours before administration of Ethanol. All pretreatments of the last dose were given orally one hour before the experiment. Gastric ulcer was induced after 60 min of *F.thonningii* in

200mg/kg doses and Misoprostol 5µg/kg treatment by administering ethanol (90%%w/v) at a dose of 1ml/200g body weight (≈0.2ml) to each animal and after 1 hr, animals were scarified with spinal dislocation; stomach was incised along the greater curvature and ulceration was scored similar to PL (77).

3.2.5.3 Ulcer healing effect on Indomethacine Induced Ulcer

This test was the first to study ulcer healing effect on since there was no well-studied model for ulcer healing study the protocol of P.Guha et al (78) was followed for ulcer induction , ulcer was induced with Indomethacine (18mg/kg) in order to evaluate the ulcer healing effect of the plant extract which was compared with negative control (vehicle) and positive(Misoprostol 5µgm/kg) treated groups. The treatment groups received CEFT 100,200,400 mg/kg (once daily). The first dose was given 6 h after induction of ulcer with indomethacine (18 mg/kg). Four days after ulcer induction, analysis has been done.

3.2.6 Parameters for Evaluation of Antiulcer Activity

3.2.6.1 Macroscopic Evaluation of Stomach

The stomach was opened along the greater curvature, rinsed with saline and running water to remove gastric contents and blood clots then the mucosa of each animal was labeled and placed on sodium phosphate buffered 10% formalin until it will examined by a 10X magnifier lens to assess the formation of ulcers. The numbers of ulcers was counted. Scoring of ulcer was based on the method by *Kulkarni* (79) as:

Normal colored stomach..... (0)
Red coloration..... (0.5)
Spot ulcer.....…. (1)
Hemorrhagic streak... (1.5)
Deep Ulcers.....… (2)
Perforation.....… (3)
Mean ulcer score for each animal will be expressed as ulcer index.
Ulcer index (UI) was measured by using following formula:
$UI = UN + US + UP \times 10^{-1}$

Where,UI= Ulcer Index; UN = Average number of ulcers per animal; US =Average number of severity score; UP = Percentage of animals with ulcers (73).

Percentage inhibition of ulceration will be calculated as below:

$$\% \text{ Inhibition of Ulceration} = \frac{(\text{Ulcer index Control} - \text{Ulcer index Test})}{\text{Ulcer index Control}} \times 100$$

$$\% \text{ Protective ratio} = 100 - \left(\frac{\text{UI pretreated}}{\text{UI Control}}\right) \times 100$$

$$\% \text{ Curative ratio} = 100 - \left(\frac{\text{UI Treated}}{\text{UI Control}}\right) \times 100$$

3.2.6.2 Determination of pH and Gastric Volume

An aliquot of gastric juice was taken and pH of the solution was measured using pH meter based on the method of Tan (54). The volume of Gastric Juice of each animal was measured after centrifugation with 1000 rpm for 10 minutes and analyzed since it is one parameter for the study of the anti-secretory effect of the plant extract.

3.2.6.3 Determination of Total Acidity

An aliquot of 1ml gastric juice diluted with 9 ml of distilled water was taken and two drops of phenolphthalein indicator was added. Then, it was titrated with 0.01N NaOH until a permanent pink color was observed. Based on the volume of 0.01N NaOH consumed, The total acidity was expressed as mEq/L by the following formula (54):

$$\text{Total Acidity} = \frac{\text{Vol. of NaOH} \times \text{N} \times 100 \text{ mEq/L}}{0.1}$$

3.2.7 Data quality control

The data quality was maintained by simple randomized grouping of animals; blinded data collection system of all parameters; maintaining and applying standard procedures and using analytical graded products.

3.2.8 Data Analysis

SPSS version 20 was used for data entry, coding, cleaning and analysis of results. The result was expressed as the mean ± SEM for each parameter. Statistical differences were evaluated using a one-way Analysis of Variance (ANOVA) followed by post hoc Tuckey's multiple comparison tests. Student Paired *t-test* was also used to compare parameter results between single and repeated administration of the same doses. Results were considered to be statistically significant at ($P<0.05$).

3.2.9 Ethical Clearance

Ethical clearance was obtained from research and ethics committee, department of pharmacology, University of Gondar with Reference number of SOP4/76/09.All procedure done on animals were done based on the rules and regulations set by a Guide for the Care and Use of Laboratory Animal (80).

4. RESULTS

4.1. Percentage yields of crude extract and fractions

The 80% hydromethanol stem bark extract of *F.thonningii* was a dark brown solid, while the chloroform fractions was a greenish black paste and the aqueous fraction reddish brown paste, a small greenish layer was observed on the foor of the beaker of N-hexane fraction. The percentage yields of the crude extract and solvent fractions are shown in Table 1.

Table 1- Percentage yield of *F.thonningii* stem bark extract (1000 gm)

Extract	Yield (gm)	Percentage yield
Crude extract	135/1000 gm bark	13.5 %;w/w
Aqueous fraction	12.0/67 gm crude	17.91 %
Chloroform fraction	5.5/67 gm crude	8.2 %
N-hexane fraction	---	Very small

4.2 Preliminary phytochemical screening

The crude bark extract of *F.thonningii* contains secondary metabolites shown on table 2

Table 2-Preliminary phytochemical screening of *F.thonningii* stem bark

Test	Phytochemical	Result
1.	Terpinoides	YES
2.	Saponins	YES
3.	Alkaloids	YES
4.	Tannins	YES
5.	Plant steroids	NO
6.	Anthraquinones	YES
7.	Glycosides	YES
8.	Phenolic compounds	YES
9.	Flavonoids	YES

4.3 Acute Oral Toxicity Studies

The crude extract of *F.thonningii* stem bark administered up to 2000 mg/kg did not cause any mortality in mice. None of the animals tested produced any gross apparent effect on general motor activity, muscular weakness, fecal output and feeding behavior during the period of observation.

4.4 Anti-ulcer Results

4.4.1 Effects of Crude Extract of *F.thonningii* on Pylorus ligation-induced ulcer

A dose dependent significant reduction in volume of gastric juice on CEFT100 mg/kg (*P*<0.01), CEFT200 and CEFT400 (P<0.001) was found and Total acidity was reduced significantly (*P*<0.001) for all three doses compared with the negative control. (Table- 3).

Table 3-Anti -ulcer (anti secretory) effect of crude extract of *F.thonningii* on pylorus ligated induced ulcer model

Group	Treatment	Dose(mg/kg)	Volume of gastric juice	Reduction of vol. (%)	Total Acidity	Reduction in acidity (%)
I	vehicle only	_	5.91±0.62	-	51.66±3.07	-
II	*F.thonningii*	100mg/kg	2.61±0.81^{a2}	55.8	30.00±2.58^{a3}	41.9
III	*F.thonningii*	200mg/kg	1.92±0.24^{a3}	67.5	23.33±2.10 ^{a3}	54.8
IV	*F.thonningii*	400mg/kg	2.16±0.30^{a3}	63.4	23.33±3.07^{a3}	54.1
V	Ranitidine	50mg/kg	0.60±0.13^{a3}	89.8	21.66±3.07^{a3}	58.1

Note: Values are expressed as Means ± S.E.M (n=6),2=P<0.01, 3=P<0.001 a=compared with negative control group(95% CI) Statistical comparisons are significant at P-Value<0.05

Gastric pH and Ulcer Index

The two doses, CEFT 200 and CEFT 400 significantly Increased the gastric pH (P<0.05) and reduce UI (P<0.05) compared to the negative control group. Extracts decreased the UI in a dose dependent manner and CEFT 200 and CEFT 400 reduced percent of UI more than the standard drug (Ranitidine) as shown in (Table-4).

Table 4-Effect of crude extract of *F.thonningii* on pH and ulcer index on PL Rats

Group	**Treatment**	**Dose**	**PH**	**Ulcer Index(UI)**	**Reduction in Ulcer Index (%)**
I	Vehicle	-	3.16±0.16	15.93±0.79	-
II	*F.thonningii*	100mg/kg	4.50±0.34	10.58±2.14	33.6
III	*F.thonningii*	200mg/kg	5.13±0.61^{a1}	6.12±2.74^{a1}	61.6
IV	*F.thonningii*	400mg/kg	5.00±0.51^{a1}	6.04±2.7^{a1}	62.0
V	Ranitidine	50mg/kg	5.37±0.30^{a1}	8.20±2.60	48.5

Note: Values are expressed as Means± S.E.M (n=6)1= P<0.05, a=Compared with negative control, (95% CI) Statistical comparisons are significant at (P<0.05)

4.4.2 Effect of *F.thonningii* 200 mg/kg on Ethanol induced ulcer

4.4.2.1 Single dose administration

No significant reduction of the ulcer score and ulcer index for all fractions of the same dose. Misoprostol 5µg/kg had reduced UI significantly (P<0.05).The percent reduction in UI was 59.1%, 43.76%, 59.0%, 73.34% for the crude, chloroform, aqueous fractions and the standard drug respectively.

Table 5-Effect of crude and solvent fractions of FT 200 mg/kg on ethanol induced ulcer model (single dose)

Group	Treatment	Dose(mg/kg)	Ulcer Score (US)	Reduction US (%)	Ulcer Index(UI)	Reduction in UI (%)
I	Vehicle(dist.water+6% Tween80)	_	0.84±0.14	-	14.67±0.99	-
II	*F.thonningii*(Crude)	200mg/kg	0.33±0.17	60.7	6.0±2.68	59.1
III	*F.thonningii*(Chloroform Fraction)	200mg/kg	0.58±0.24	30.9	8.25±2.61	43.76
IV	*F.thonningii*(aqueous Fraction)	200mg/kg	0.58±0.30	30.9	6.08±2.72	59.0
V	Misoprostol	5µg/kg	0.25±0.17	70.24	3.91±2.47[a1]	73.34

Note: Values are expressed as Mean ± S.E.M (n=6) 1=P<0.05;a=Compared with vehicle (95% CI) Statistical comparisons are significant at (P<0.05)

4.4.2.2 Repeated dose administration

Both the Hydromethanolic and aqueous fractions reduced UI significantly (P<0.05).while chloroform fraction did not show significant reduction in UI(P>0.05).The percent reduction in UI was 39.76%,70.76%,71.86%,72.96% for chloroform fraction, aqueous fraction, crude extract of *F.thonning*ii and Misoprostol(standard) on respective ascending orders.(Table-6).

Table 6-Effect of crude and solvent fractions of FT 200mg/kg on ethanol induced ulcer (10 days)

Group	Treatment	Dose(mg/kg)	Ulcer Score (US)	Reduction US (%)	Ulcer Index(UI)	Reduction in UI (%)
I	Vehicle(dist.water+6 %Tween80)	_	0.73±0.08	-	15.39±0.72	-
II	*F.thonningii*(Crude)	200mg/kg	0.5±0.31	31.50	4.33±2.74[a1]	71.86
III	*F.thonningii*(Chloroform Fraction)	200mg/kg	0.61±0.27	16.43	9.27±2.98	39.76
IV	*F.thonningii*(aqueous Fraction)	200mg/kg	0.5±0.31	31.5	4.5±2.8[a1]	70.76
V	Misoprostol	5µg/kg	0.33±0.24	54.8	4.16±2.6[a1]	72.96

Note: Values are expressed as Mean ± S.E.M (n=6) 1=P<0.05;a=compared with vehicle group, (95% CI) Statistical comparisons are significant at (P<0.05)

There was no statistically significant deference on US and UI between single and repeated dose of the same group when analyzed by paired-sample T-test for all fractions.

4.4.3 Assessment of healing on Indomethacine Induced ulcer

There was a dose dependent reduction UI (61.72%.61.15%,63.35%;for CEFT 100, 200,400 mg/kg respectively) but no significant reduction on US and UI for CEFT 100,200 and 400 mg/kg except Misoprostol (P<0.05) (table-7).

Table 7-Ulcer healing effect of CEFT100, CEFT200 and CEFT400 on indomethacine induced ulcerated mice (4 day treatment)

Group	Treatment	Dose(mg/kg)	Ulcer Score (US)	Reduction US (%)	Ulcer Index(UI)	Reduction in UI (%)
I	Vehicle(dist.water+6% Tween80)	_	0.65±0.15	-	10.45±2.1	-
II	*F.thonningii(crude)*	100 mg/kg	0.16±0.10	75.4	4.00±2.50	61.72
III	*F.thonningii*(Crude)	200mg/kg	0.25±0.17	61.5	4.06±2.58	61.15
IV	*F.thonningii*(Crude)	400mg/kg	0.16±0.10	75.4	3.83±2.42	63.35
V	Misoprostol	5µg/kg	0.00±0.00^{a*}	100	0.00±0.00^{a*}	100

Note: Values are expressed as means ± S.E.M (n=6)*P<0.05; a=Compared with negative control group; (95% CI) Statistical comparisons are significant at P-Value<0.05

5. DISCUSSION

This study has been done to evaluate the anti-ulcer effect of *F.thonningii* stem bark considering the global prevalence of the disease and the little attention given to search novel antiulcer agent. the 80% Hydromethanolic solvent is the most preferred one to dissolve both polar and non-polar phytochemicals since many organic substances are not extracted from aqueous solvents only (81).

The present study's Preliminary Phytochemical screening result revealed presence of: Tannins,Saponin, Anthraquinons,Terpinoids,Phenoliccompounds, Flavonoids and cardiac glycosides the result was coherent with reports of other Pharmacological researches in Zimbabwe and Nigeria(58, 61).However the phytochemical result of this study was in contrast with the taxonomical report in Nigeria (82). The difference may be due to the aim of the research, the solvents used, soil or climatic factors. methanolic extract of stem bark of *F.sycomorous* which is grouped in the same family (Moraceae),had the above metabolites and absence of plant steroids (70).

The above phytochemicals in the study plant have anti-ulcer activity especially Phenolic compounds and Flavonoids that possess an ideal structural chemistry for free radical scavenging and Antioxidative properties. As known, this effect when combined with endogenous gastric peroxidases,can reduce free radical accumulation and prevent oxidative mucosal damage. The effect may arise from their high reactivity as electron donors (83) Saponins have anti-ulcer action by mediating the formation of mucous (84).Tropane alkaloids*(Anisodamine* and *anisodine)* which are analogs of atropine block the muscarinic activity of acetylcholine and show antisecretory effect.Quinolizidine alkaloids (*matrine*, *oxy-matrine)* and Indole alkaloids (*Nigakinone* and *methylnigakinone)* have reported antiulcer effects by decreasing the gastric acid/pepsin secretions and increase the protection of the mucous membrane (85)**.**Flavonoids can also increase mucosal prostaglandin, decrease histamine secretion by the inhibition of *Histidine Decarboxylase*,improve mucus secretion and inhibition of *H. pylori* growth. Tannins can prevent ulcer by improving vasoconstricting effects (86) and they have astringent properties by reacting with the layer proteins and are known to 'tan' the outermost layer of the mucosa to render it less permeable to chemical injury or irritation and Saponins activate mucous membrane protective factors(87).The study

plant was rich on the above mentioned phytochemicals and anti- ulcer effect might be due to the presence of one or more of these secondary metabolites.

The models for the present study have been selected based on the multiple Pathophysiology of PUD considering Central and peripheral factors (23, 88) as well as the actions of prostaglandins on the disease (23).Pylorus ligation (surgical),an exogenous ulcerogen, ethanol (chemical) and prostaglandin synthesis inhibiting, indomethacine (pharmaceutical) had been selected. In pyloric ligation,the principle of ulcer formation is: accumulation of gastric juice and pepsin which involve in the auto-digestion of gastric mucosa implicated to ulcer incidence (52). Stress due to distension of stomach tissue increases gastric acid secretion and/or stasis of acid and fluid increases volume of secretion which is also an important factor in the formation of ulcer due to exposure of the unprotected lumen of the stomach by the accumulated acid. This model is simple, reproducible and highly predictable which never utilizes exogenous ulcerogen and suitable for evaluation of antiulcer drugs with anti-secretory mechanisms (73).The present study showed a significant dose dependent reduction in volume of gastric juice on CEFT100 mg/kg (P<0.01), CEFT200 and CEFT400 (P<0.001) as compared with the negative control. Result was comparable to the standard drug Ranitidine 50 mg/kg. With percent reduction in gastric volume of (55.8%, 67.5%, 63.4% in respective doses vs 89.8% standard. Ulcer Index in the negative control group was almost similar with other studies of the same procedure(49, 77) indicating that the study was done according to the standard procedures. While gastric juice volume was lower (5.91 ml versus 8.1ml) this deference may be due to the time of administration (1 hr versus 45 minutes) which is related with onset of action of phytochemicals.

All the three doses CEFT 100, CEFT200, CEFT400 significantly decreased total acidity (P<0.001).The study result confirmed the literatures' reports as even complete elimination of acid secretion and the concept of mucosal cytoprotection (23). The dose dependent antiulcer activity was further supported by ulcer index on CEFT 200 and CEFT 400 (P<0.05) while CEFT 100 and even ranitidine 50 mg/kg did not have significant effect on UI(P>0.05) though it reduces acid secretion significantly.From this result one can see that antisecretory drugs only or acid suppressive therapies may not be necessarily effective on the rate of tissue regeneration (23) as this scenario of ranitidine was reported on previous studies (49) there was also significant change in pH (P<0.05) on CEFT200 and CEFT400 (76).This effect

suggests that the extract's anti-ulcer effect is mainly due to its antisecretory in complement with cytoprotective effect or mucosal tissue regeneration of the plant due to presence of the active Phytochemicals.

No significant reduction of the ulcer index for all fractions in Single dose treatment ($P>0.05$) on ethanol induced ulcer.

While, repeated doses of crude extract(200 mg/kg) showed better reduction in UI(59.1% for 1 day and 71.86% for 10 days compared to their respective negative control groups);chloroform fraction of *F.thonningii* (200 mg/kg) reduced UI (43.76 % for 1 day and 39.76% for 10 days) and the aqueous fraction (200mg/kg) reduction in UI (59 % for 1 day and 70.76% for 10 days).Repeated doses of crude extract and aqueous fractions have shown a statistically significant reduction in ulcer index ($P<0.05$) equivalent with Misoprostol (72.96%) the present study was coherent with the report of the same family plant *F.arnottiana* methanolic leaf extract 500 mg/kg for 10 days in Portugal, with reduction in UI of 63% (89).The present study also affirms the importance of repeated dose administration reported by previous studies (75, 76).The study justified the pharmacokinetic concept of the active principle that the plant extracts having active ingredient need a repeated dose administration to attain its therapeutic window and steady state for ulcer protective effect.

From different solvents point of view, hydromethanolic extract and aqeoues fractions have showed a statistically significant reduction on ulcer index ($P<0.05$).While Chloroform fraction of FT200 did not ($P>0.05$) (Table-5).From this finding we can conclude that phytochemicals found in *F.thonningii* which have mucosal cytoprotective activity can be solublized by relatively polar solvents (water, methanol) than weakly polars (chloroform) as anti-ulcer effect of the aqueous leaf extract was also reported by other study(90).

Ethanol-induced ulcers in mice are characterized by heavy bleeding since it can cause immediate stasis in the blood flow (91) Studies show that (50%w/v) ethanol can induced gastric damage in >90% of test animals (92) and lesions are blackish of varying size parallel to the major axis. Lesions can be inhibited by various drugs like Misoprostol.since ethanol can cause turbulences in gastric secretion, alter in the permeability of the gastric membrane, free radical production during its metabolism as oxygen derived free radicals have been found in the mechanism of acute ulceration in the gastric mucosa leading to increased intracellular membrane permeability to sodium and water and enormous intracellular increase

of calcium that corresponds to the pathogenesis of gastric mucosal injury (93).plant principles which prevent ulcer can have cytoprotective effects in general.

The result on ulcer healing effect showed all doses (CEFT100, 200,400 mg/kg) did not have significant effect (P>0.05).Here many things can be rised starting from the ulcer induction protocol as: indomethacine at 18 mg/kg cased ulcer score of 0.65±0.15;which was lower as compared with the report done in Addis Ababa university (dose 28 mg/kg ;ulcer score;1.83±0.17) (88).Meanwhile, a dose-dependent anti- ulcer activity was recorded in the indomethacine model which indicated the plants low potency on ulcer healing effect that may need a higher dose to produce full protection against the ulcerogenic effect of NSAIDs.Simiar study on The plant *Dialium guineense* reported that antiulcer effect recorded only by the highest dose of 750 mg/kg (94).

As known,inhibition of prostaglandin synthesis by blocking the enzyme cyclooxygense (COX) with NSAIDs which are the second most common etiologic agents of ulcer next to *H.Pylor,*can increase susceptibility of GI mucosa by luminal irritants, alter the micro circulation that is critical to the pathogenesis of ulceration and has negative impact on mucus and bicarbonate secretion (95).The present study result we can also lead to suggest that,either the anti-ulcer activity of *F.thonningii* may not be related with modulation of prostaglandin synthesis or non selective inhibition of the constitutive enzyme COX-1 to an inflammatory enzyme(COX-2) as inhibition of COX-1 can delay healing by reducing platlates aggregation and even it can cause mucosal bleeding. this assumption was set from the report on anti-inflammatory and prostaglandin inhibition activity of the plant (61) eventhough ulceration could be induced at day 4 after induction with indomethacine 18 mg/kg (78),higher than this dose might be used as a protocol for ulcer healing study**.** This test was the first to study ulcer healing effect by treating animals after induction of ulcer we tested the protocol since there was no well-studied model for ulcer healing study.

Hydromethanolic extract of *F.thonningii* bark did not show any sign of acute toxicity up to 2000mg/kg suggesting that stem bark of the plant is relatively non-toxic and same result has been reported on the leaf extract of *F.thonningii* in Nigeria by other studies(61, 96) and fruit is reported as edible in the study area(97).

6. CONCLUSION

The present study demonstrates that the crude extract and aqueous solvent fraction of the stem bark of *F.thonningii* has an anti-ulcer effect, which may probably be related to the anti-secretory, mucosal protecting, anti-inflammatry or free radical scavenging activity of the phytochemical constituents reported. This study justifies the traditional claims on the folk medicine reported in Ethiopia and Nigeria. This plant extracts also exhibited safety profile at the maximum dose of 2000 mg/kg. Therefore, the aqueous fractions and Hydromethanolic extract could represent a new source for the development of new plant based antiulcer agent.

7. RECOMMENDATIONS

Pharmacological Screening studies are bases for the progress and expansion of researchable areas on the study of new therapeutic agents to get novel therapeutic agents for different ailments. In order to achieve these aims, studies need integrated supports from multidisciplinary stakeholders.

From the present study, the following works are suggested for further investigation on the plant.

- ✓ Mechanism of action for antiulcer effects.
- ✓ Further quantitative phytochemical investigation to identify and quantify the

Active anti-ulcer component/s from the plant.

- ✓ well-designed NSAID induced ulcer model for analysis of ulcer healing effect by treating medications after induction of ulcer

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9. ANNEXES

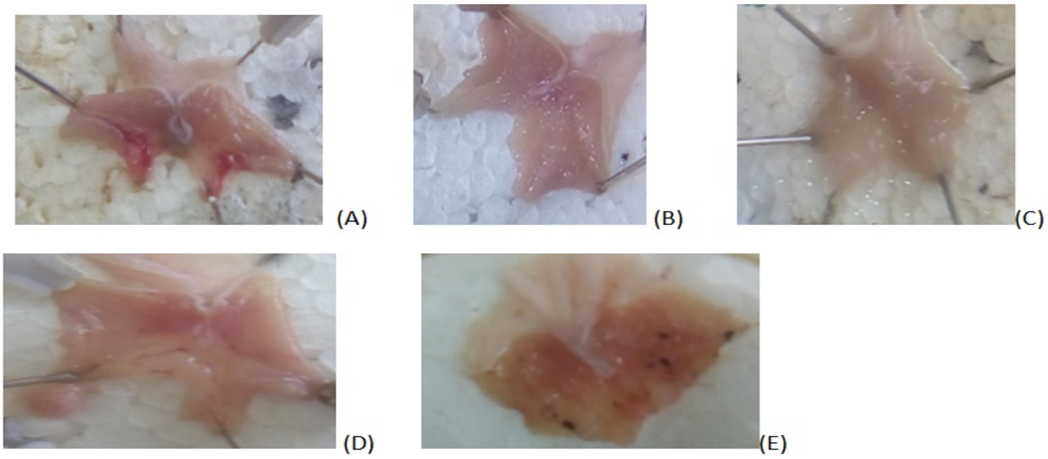

Figure 3- Effect of *F.thonningi* bark extract on ethanol induced ulcerated mice.

(A) Negative control (distilled water+4% tween 80 for 10 days);(B) Treated with standard drug (Misoprostol 5mcg/kg,10days);(C) Treated with CEFT200(10 Days);(D) Treated with Aqueous fraction of FT200 and (E)Treated with Chloroform fraction of FT200(10 Days)

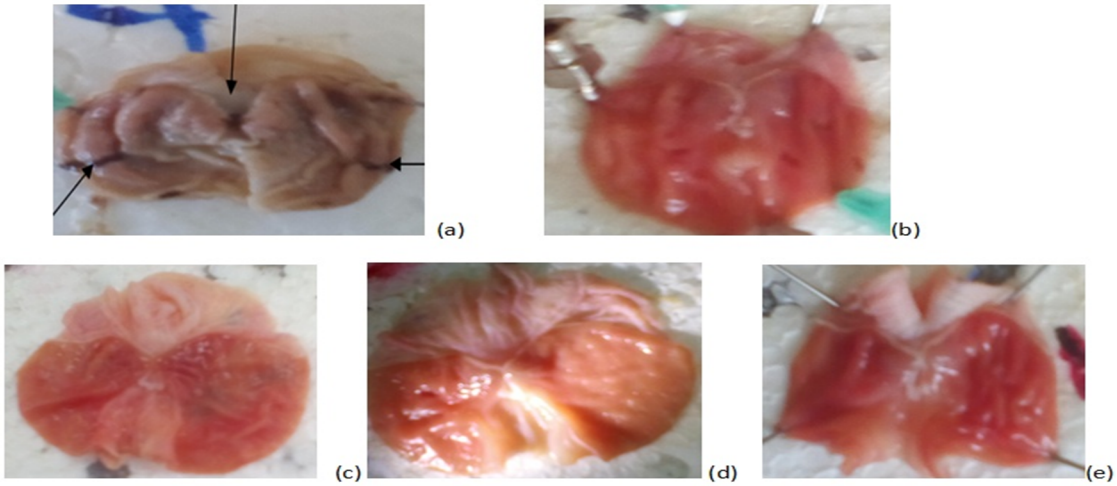

Figure 4-Efffect of *F. thonningii* on pylorus ligated Rat mucosa pretreated for 10 days: vehicle only (a):CEFT100mg/kg(b),CEFT200 mg/kg(c),CEFT400 mg/kg(d) and Ranitidine 50 mg/kg(e)

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SCHOOL OF PHARMACY

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This is to certify that the research thesis prepared by Mr. HabtalemAdane entitled: "***Evaluation of Anti- Ulcer Effect of Hydromethanolic Crude Extract and Solvent Fractions of the Stem Bark of Ficus thonningii on rodent Models"*** and submitted in partial fulfillment of the requirements for the degree of Master of Science in Pharmacology complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Approved and signed by the investigators and Advisors:

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Advisor	Signature	Date
Dr. Digambar Ambikar (M.Pharm, PhD)	_____	_____
Co-advisor	Signature	Date
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Investigator	Signature	Date